stomach and intestines of a Cobra suffering from gastro-enteritis, and made the following remarks :—

"These specimens are shown in order to call attention to the condition of inflammation of the stomach and intestines in snakes from the Society's Collection. Out of 160 cases examined during the last year by far the larger number showed this condition in varying degrees; and that is after excluding those cases due to the direct irritation of worms which had caused ulceration or perforation.

"Of course there are many causes of inflammatory conditions of the stomach and intestines, but it would appear, from the large percentage of cases showing this condition, that there must be some common cause, and as the present method of feeding the snakes is an unphysiological one, it might be worth while to consider whether it may not be the cause of the large mortality from these inflammatory conditions of the alimentary tract."

The following papers were read :—

1. Observations on the Flagellates Parasitic in the Blood of Freshwater Fishes. By Prof. E. A. MINCHIN, M.A., V.P.Z.S.

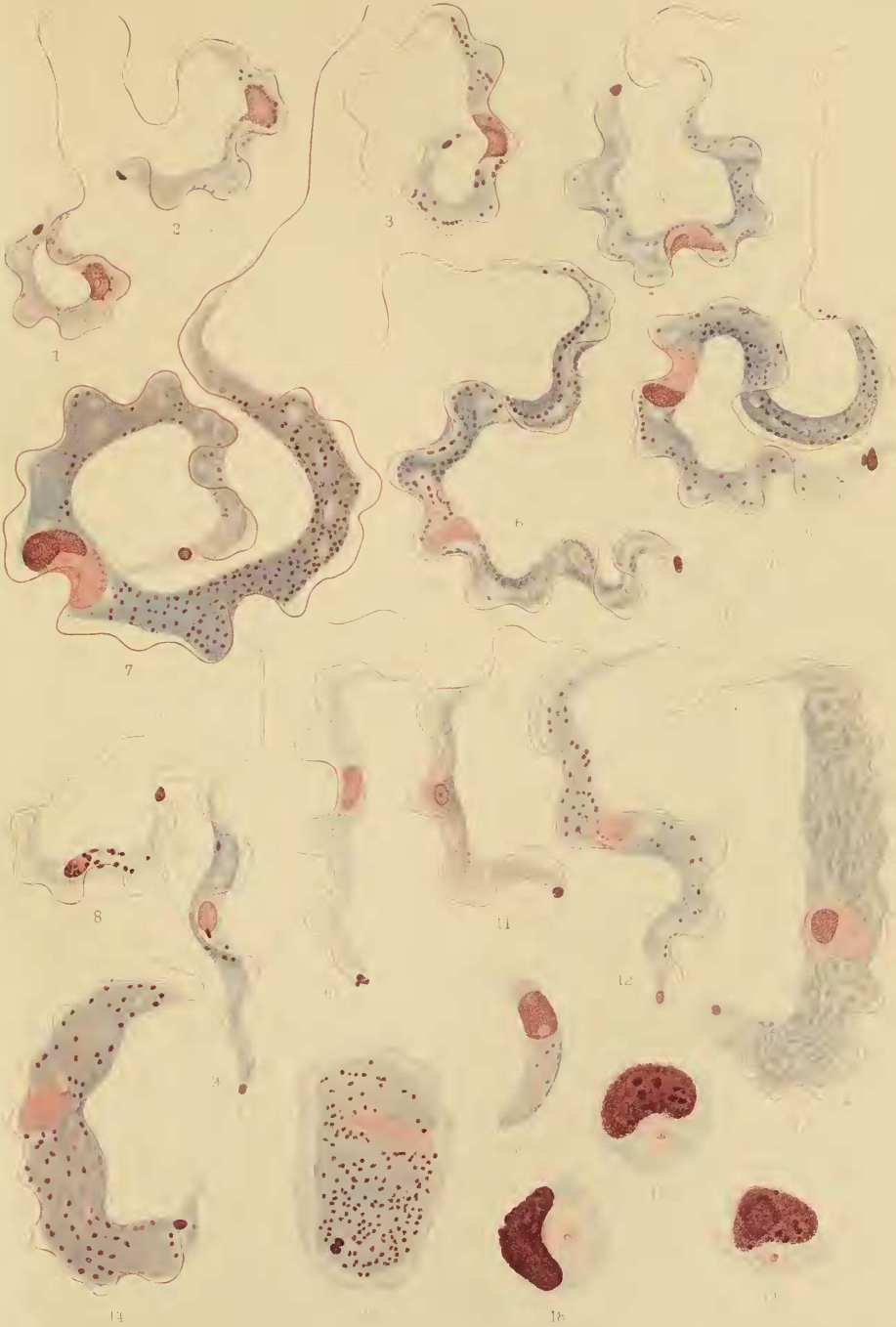
[Received December 23, 1908.]

(Plates I.–V.*)

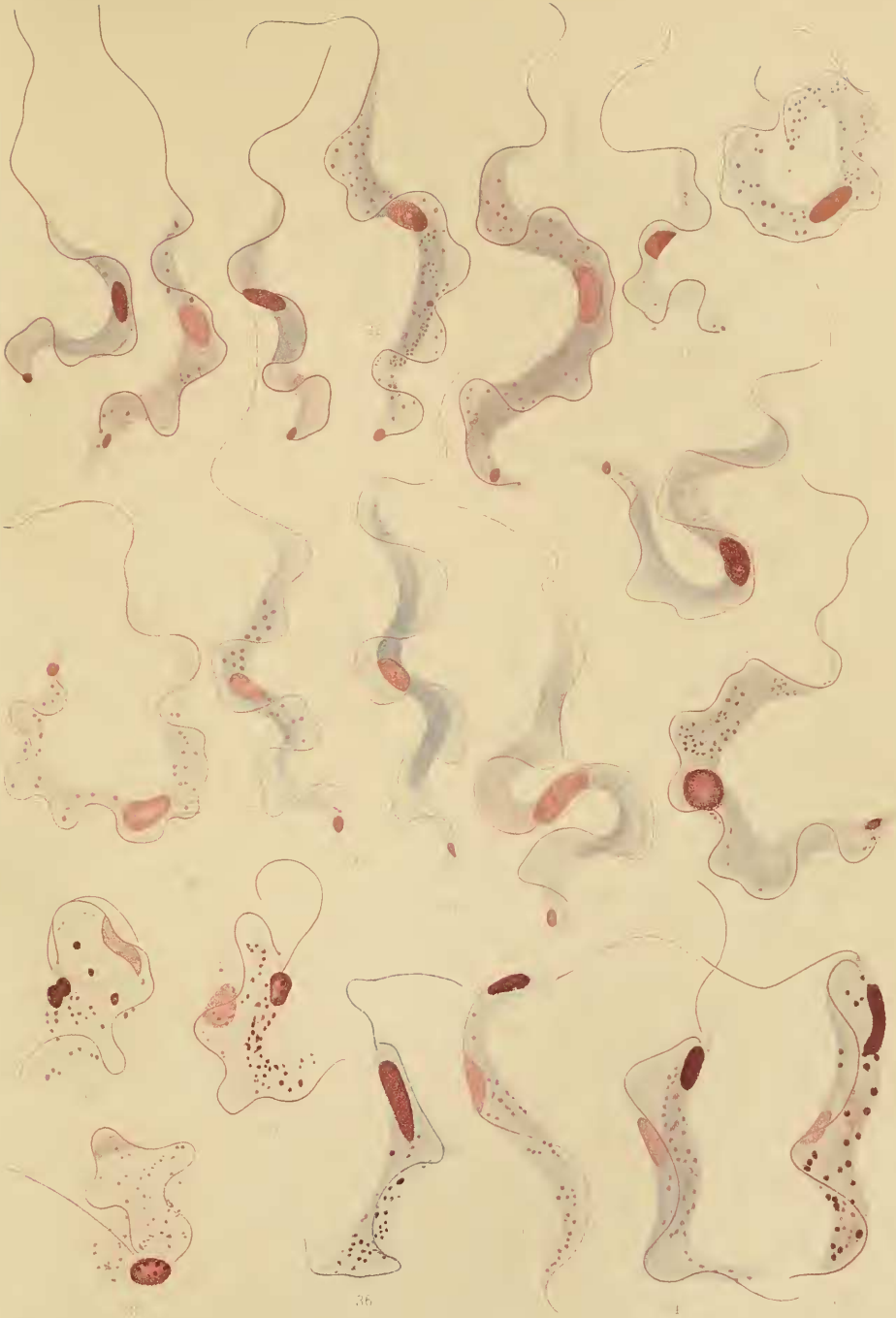
Introductory.

The trypanosomes and trypanoplasms of freshwater fishes have been studied by a number of naturalists abroad, and have been the subject of several important memoirs, more especially by Laveran & Mesnil, Léger, and Brumpt in France, and by Keysselitz in Germany. In this country, however, little attention has been paid to them. I was therefore glad to avail myself of the exceptional opportunities offered by the Sutton Broad Laboratory, to study the parasites of the fishes in the Norfolk Broad. I desire to take this opportunity of expressing my warmest thanks to my friends Messrs. Eustace and Robert Gurney for their kindness in putting at my disposal the resources of their picturesque and well-equipped laboratory, and for much help during my stay there. I spent portions of my summer vacations at the laboratory, about three weeks in August and September 1907, and five weeks in the same months in 1908, and during these periods I occupied myself almost entirely with these parasites. Although I have only touched the fringe of the question so far, and there is still everything to be discovered and worked out concerning the all-important

* For explanation of the Plates see p. 29.



TRYPANOSOMES OF EEL AND PERCH.



TRYPANOSOMES OF PIKE TENCH AND BREAM.
TRYPANOPLASMS OF PIKE.

M. R. ad nat. del.

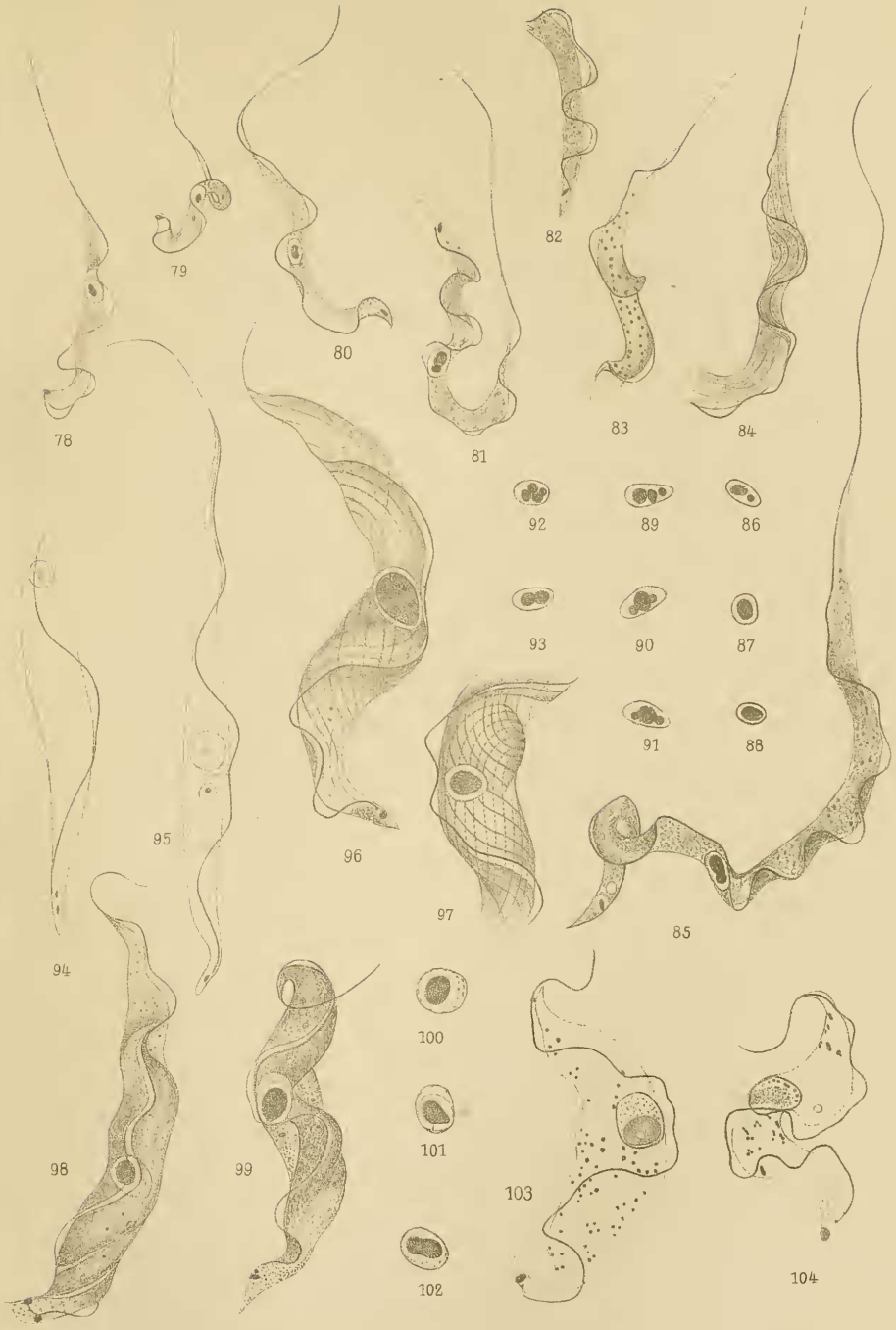
Lith. Anst. v. E. A. Funke, Leipzig.

TRYPANOPLASMS OF TENCH, BREAM AND RUDD.

M. R. ad nat. del.

Lith. Anst. v. E. A. Funke, Leipzig.

TRYPANOPLASMS OF PIKE, TENCH AND BREAM.
TRYPANOSOMES OF TENCH



L. A. M. & M. R. ad. nat. del.

Lith. Anst. v. E. A. Funke Leipzig.

TRYPANOSOMES OF EEL AND PERCH.

question of the transmission of these parasites, I thought it worth while to publish my investigations at the point they have reached, leaving the subject in a condition in which it can be taken up again and carried further by myself or by anyone else working in the same region.

Methods and Technique.

In examining the blood of fishes for the parasites, it is not difficult, when dealing with a fish of fair size, to take the blood from the gills of the fish without materially injuring it. There is always a risk, however, that the blood obtained in this way may contain a certain amount of water mixed with it. To obtain good smears I found it best to sacrifice the fish. Having killed it by a smart blow on the head with a blunt instrument (best a stout rod of hard wood) I opened up the pericardial region and took the blood from the heart by means of a fine capillary glass tube, thrust through the wall of the heart; the blood was allowed to run up into it, and then blown on to the slide or coverslip. The whole process has to be done quickly, since fish-blood coagulates very rapidly. Fish which are used in this way for making blood-smears suffer no detriment in respect to their culinary properties and need not be wasted.

Much has been written lately about methods of fixation of these blood-parasites, especially with regard to the procedure most in vogue, of drying smears of the blood. That the process of drying affects the minute structural details cannot be doubted and can be demonstrated easily. It should, however, be pointed out that the effects of drying, so far as trypanosomes are concerned, differ greatly according as the drying is done before, or after, fixation with some histological reagent. Very instructive in this respect are the species of the genus *Trypanoplasma*. Their soft protoplasmic bodies become greatly deformed if dried before fixation; in this respect they contrast strongly with the species of *Trypanosoma*. In some fishes, for example the pike, tench, and bream, it is common to find both trypanosomes and trypanoplasms in the blood; in smears dried before fixation, the trypanosomes may be found quite satisfactory in form and general structure, while the trypanoplasms side by side with them are deformed almost beyond recognition; compare figs. 25, 26, and 39, showing trypanosomes and trypanoplasms of the pike, from the same slide; figs. 32, 53, 54, showing similar conditions in the blood of the bream. In some cases, however, a trypanoplasm may, apparently, flatten down evenly and thus give a fairly reliable representation of its natural form and structure (compare figs. 37 and 38, from the same slide); such cases, however, are in my opinion to be regarded as accidental and exceptional, and the rule is that trypanoplasms when dried become much deformed. It is therefore a matter of astonishment to me that Keysselitz should have relied so much on material dried before fixation for his

lengthy and detailed study of the life-history of *Trypanoplasma cyprini* ("*borreli*"). It is, perhaps, for this reason that Keysselitz was unable to distinguish more than one species of *Trypanoplasma*.

The deformation subsequent on drying is entirely avoided if the smear be placed as quickly as possible, immediately after smearing and before any appreciable amount of drying has taken place, into a stoppered tube or bottle containing a few drops of osmic acid, 4% solution. It should be exposed to the vapour for 30–60 seconds, and then transferred, with or without drying, to absolute alcohol. After the fixation with osmic vapour the drying does not deform even the trypanoplasms, and the natural form, size, and appearance of these flagellates are, in my opinion, better preserved by this method than by any other. The osmic-fixed smears stain well with Giemsa's stain, but are apt to stain rather too darkly and, in the case of large fleshy forms of the parasites, to become very opaque. Speaking generally, they should be stained for a much shorter time than the preparations dried off before fixation.

The weak point of the preparations stained by the Romanowsky method, whether dried before or after fixation, or not dried at any time, lies in its effects on the nuclear apparatus. I hope to discuss this point in greater detail in a memoir which I am preparing on the structure of *Trypanosoma lewisi*. I will say here only that the Romanowsky stain, in its various modifications, gives results with regard to nuclear structure which are not capable of uniform interpretation, and which, in my opinion, are untrue and misleading. I believe the defects of the stain to be due principally to the fact that the red dye or dyes in the stain are precipitated not only in, but around, certain objects in the preparations; with the result that the kinetonucleus, for example, appears many times its real size, while in the trophonucleus minute, almost ultramicroscopic granules become enlarged to coarse granules obscuring the true structure. This effect is not due to the drying, since it is to be observed in preparations stained by the Romanowsky method and mounted in Canada balsam without being dried at any stage of the process. I believe, however, that the process of drying, whether before or after fixation, may and does add greatly to the falsity of the nuclear results obtained by the stain. While osmic vapour fixes very perfectly the cytoplasmic portions of the body, it apparently leaves the nuclear constituents unfixed and in a fluid condition. Hence, while the general structure of the body is very perfectly preserved, even when dried, after osmic fixation, the nucleus remains in a fluid state and liable to deformation. That, at least, is the impression which a comparison of different methods gives me.

For interpretation of the results obtained by the Romanowsky stain, it is very instructive to compare the totally different results obtained by staining the same objects with Heidenhain's iron-hæmatoxylin. For this it is necessary, however, that the smears should be suitably fixed and that they should have never been

dried. I have tried various fixatives and get the best results with sublimate-acetic ($HgCl_2$ saturated in water 95 volumes, glacial acetic 5 volumes), Schaudinn's fluid ($HgCl_2$ saturated in water 2 volumes, absolute alcohol 1 volume, with addition of a few drops of glacial acetic), and Mann's picrocorrosive-formol solution. The first two fixatives may be used directly on the wet smears, or with previous exposure to osmic vapour for a short time. I find it useless, however, to fix the smears on slides; the reason being that it is impossible, or at least very difficult, to put them into the fixatives without making them dive in with one end foremost, a process which causes distortion in the wet and still unfixed elements of the smear. Satisfactory preparations can only be obtained by these methods of fixation, by making the smear on a coverslip and dropping it at once with the smear downwards into the fixative. I hold the coverslip with the fingers of my left hand, and a glass rod in my right hand; a drop of blood is placed by an assistant on the coverslip; I then smear it out immediately with the glass rod and drop it instantly into the fixative. The whole process can be done most expeditiously, and with much less risk of partial drying than when dealing with slides.

In staining trypanosomes with iron-hæmatoxylin, I find it best to let the mordant and stain act for a long time. The objects are first left in the iron-alum solution ($3\frac{1}{2}$ %) over night; they are then, after brief and rapid washing with distilled water, transferred to the hæmatoxylin solution ($\frac{1}{2}$ %) for at least 24 hours. The whole art of the process lies in the differentiation and extraction of the stain. My method is to put the coverslip into the iron-alum until colour is seen to be coming out; then the coverslip is dipped into tap-water to stop the extraction of the stain, and examined with moderate magnification (Zeiss Oc. 4, Obj. D). If the karyosome can be seen sharply and clearly in a trypanosome, the extraction is sufficient; if not, the coverslip is put into iron-alum again for a short time. Usually I do several smears of each sample of blood with different degrees of extraction of the stain. In most smears there are also thicker and thinner portions, and it is found that in thicker portions of the smear the stain is not so quickly extracted from the trypanosomes as in the thinner portions. Different degrees of extraction of the hæmatoxylin have their uses in showing up different points of structure. When the trypanosomes first come out of the stain they have an even, opaque black colour. I find that usually the stain is extracted first from the cytoplasm generally, then from the myonemes; next from the coarse granulations of the cytoplasm; next from the flagella and the blepharoplasts; next from the karyosome, and last of all from the kinetonucleus which appears to give up the colour at its periphery first. There are exceptions, however, to this order, for in some trypanoplasms (e. g. *T. gurneyorum*) the cytoplasmic granules retain the stain as long as the karyosome. In one of my preparations which was under-extracted, I found that the trypanosomes and trypanoplasms showed their form and

the flagella very sharply, but were too opaque for internal structure; I therefore had the parasites drawn in outline with the camera lucida, then unmounted the coverslip, extracted more of the stain, and, after mounting it again, added the details of minute structure to the drawings already made.

With use of iron-hæmatoxylin in never-dried preparations suitably fixed, absolutely uniform results are obtained with regard to the nuclear structure. When two such staining methods as the Romanowsky stain and the Heidenhain stain give conflicting results in matters of nuclear detail, no one with any knowledge of cytological methods would hesitate, I think, to regard as more reliable the evidence yielded by the Heidenhain stain.

The figures illustrating this memoir were drawn with the camera lucida to a magnification of 2000 linear, with the exception of the sketches drawn from living trypanosomes (figs. 77, 94, 95). The majority of the drawings were executed by my assistant Miss Rhodes, to whom I desire to express my thanks for her skilled help in this work.

General Remarks on the Trypanosomes and Trypanoplasms of Fishes.

It would, perhaps, be more logical if the general account of these parasites came after the detailed descriptions of the species, since the conclusions at which I have arrived are founded on the data which are set forth in the special descriptions. But to many the general summary of results that is given here will be of greater interest than the, perhaps, rather wearisome special details, which therefore I relegate to second place, for purposes of reference for those specially interested.

From a study of the trypanosomes of fishes in the fresh, living condition in the blood, I have come to the conclusion that there are two types of movement: a conclusion which may perhaps be true of trypanosomes universally. Sometimes they may be seen to move in a definite direction; at other times they are seen to be twisting about in one spot without moving from it. I term these two types of movement travelling and wriggling respectively. They travel usually with the flagellum forwards; in the trypanosome of the eel I have observed progression of this type only. Some trypanosomes, however, can be seen to travel occasionally with the flagellum directed backwards; progression in this manner appears to me to occur chiefly when the trypanosome is forcing its way through corpuscles, and never when its path is unobstructed. In either case, however, progression appears to be effected chiefly by flexions of the whole body and by rippling movements of the undulating membrane, and scarcely at all by means of the free flagellum.

In wriggling movements the trypanosome simply twists over and over in S-like curves in one spot, and often appears as if tied in a knot; this is what I understand Laveran and Mesnil to mean

by the expression "pelotonné." The object of these movements is probably simply to increase the animal's power of absorbing nutriment &c. from the surrounding medium, by incessantly changing the surface of contact between the body and the blood-plasma, just as a *Tubifex* in the mud wriggles incessantly in order to bring the surface of the body into contact with fresh water and oxygen. I think it highly probable that when these parasites are being carried round passively in the circulation they perform wriggling movements only, having no occasion to travel. On the slide, however, they are seen sometimes to travel, sometimes to wriggle; but when they become moribund they only wriggle.

Trypanoplasma, so far as I have observed, travels always with the anterior free flagellum forwards; I have never seen it go with the posterior flagellum, that is to say the flagellum which runs along the edge of the undulating membrane, directed forwards. If the kinetonuclear extremities of *Trypanosoma* and *Trypanoplasma* are to be considered homologous, then the direction in which the former usually travels is the opposite to that in which the latter invariably progresses. I have not observed in *Trypanoplasma* anything comparable to the wriggling movements of *Trypanosoma*; the former genus has the body relatively much shorter and less flexible. It can be observed frequently, however, that a *Trypanoplasma* when travelling in a certain direction will quite suddenly bend over on itself and travel in a direction more or less the opposite to that which it took formerly.

A remarkable feature of some trypanosomes and trypanoplasms of fish is the great disparity in size between different individuals in the same blood. This point has already been noticed by previous observers with regard to the trypanosomes of the pike and the eel, both of which have been divided, each into two varieties, distinguished as var. *parva* and var. *magna* respectively. In the five fish-trypanosomes examined by me, I find the state of things different in different species. In the trypanosomes of the bream and the tench, which are perhaps one and the same species (figs. 27–32), I could not find any variation in size sufficiently well marked to be characterized as true dimorphism; but my material of these two forms is not so abundant as in other cases. In the trypanosome of the pike (*T. remaki*, figs. 20–26) I found the well-marked dimorphism described by Laveran and Mesnil, and though the two forms *parva* and *magna* each vary slightly in size and other characters, they were nevertheless easily distinguished, and no forms could be found transitional between them. In the trypanosome of the perch (*T. percæ*, figs. 8–14) I found three principal types—small slender forms, large stout forms, and intermediate forms. The small forms and the intermediate forms are connected by transitions (figs. 10, 11), but the stout forms stand rather apart, owing to the shortness of the free flagellum (figs. 13, 14). Finally in the eel, I found every possible gradation between the smallest and the largest forms (figs. 1–7); there is a very great difference in size between the two extremes, but the absence of a dividing line

between them makes it difficult, if not impossible, to distinguish the varieties *parva* and *magna*.

The significance of this dimorphism or polymorphism is not clear, and must be explained from the life-history. Two possible explanations present themselves: first, that the difference between small and large forms is one of growth and development; secondly, that it is a manifestation of sexual differences, small male and large female forms being differentiated from an indifferent or intermediate form. The trypanosomes of the pike and the perch rather favour the sexual hypothesis, but the state of affairs in the eel-trypanosome strongly suggests stages of growth merely. Certain facts that I have observed in the perch have awakened in me the suspicion that these fish-trypanosomes have some form of multiplication in the internal organs of the fish, and that fission of the type familiar in other trypanosomes perhaps only occurs after a new infection, just as in *Trypanosoma lewisi* fission is only found in the first week or ten days after inoculation. Fission has very seldom been seen in fish-trypanosomes; so far as I am aware, it has only been seen in *T. remaki* immediately after inoculation into a pike (Laveran and Mesnil) and in *T. granulosum* in cultures *in vitro* (Lebailly, França). The absence of fission-stages in the blood of fish infected naturally is very striking. The subject is one requiring renewed investigation.

In the trypanoplasms of the tench (figs. 40–43) and pike (figs. 33–37, 56) I have also observed smaller and larger forms, sharply distinct not only in size but even in nuclear structure; the large forms are much less common than the smaller "ordinary" forms, and both types are divisible into two categories by differences in the nuclear apparatus, especially the kinetonucleus. According to Keysselitz the large forms are gametes destined to conjugate in the intestine of the leech; those with larger kinetonuclei are regarded by him as gametes of male character, while the forms with smaller kinetonuclei are regarded as female. In one of the ordinary forms of the trypanoplasm of the pike I have seen the only example that has come under my notice, of what is apparently nuclear division (fig. 62).

It is a moot point, how far different species of trypanosomes can be distinguished by morphological characters. As I have already said, the trypanosomes of the five species of fish studied by me belong, in my opinion, to four species, namely (1) the trypanosomes of the bream and tench, (2) of the pike, (3) of the perch, (4) of the eel. Each of these four putative species can be easily distinguished from the other three by its structural characters, considered as a whole; I need only refer to my figures. There is, however, always the possibility to be borne in mind that one and the same species of trypanosome, when inoculated into distinct species of fish, might vary in character in response to differences in the environment; this is a point on which experimental evidence is needed. The trypanoplasms of the tench, bream, and pike seem to me also to constitute three well-

characterized morphological species, each quite distinct from the only specimen of *T. borreli* of the rudd that I have seen.

Considering the great variability in size of these parasites, it is remarkable that so many authors should base their specific descriptions so largely on measurements. A naturalist, for instance, of the attainments and experience of my friend M. Brumpt (to whom I owe my first lesson in the art of staining trypanosomes in blood-films) can hardly have thought such measurements to be of any real validity as specific characters.

In considering the minute structure of these parasites we may begin with the nuclear apparatus. As I have stated above, the use of iron-hæmatoxylin as a stain permits of a uniform interpretation of the appearances; and I think it is a very important point, that it is possible to give in general terms a description of the nuclear apparatus which will apply to trypanosomes and trypanoplasms alike. In both cases we find a kinetonucleus and a trophonucleus, the first named being relatively very large in the trypanoplasms, small in the trypanosomes. Near the kinetonucleus are found the minute blepharoplasts or basal granules of the flagella, two in *Trypanoplasma*, one in *Trypanosoma**.

The kinetonucleus in both cases appears as a dense body stained a uniform deep black after iron-hæmatoxylin. I have not been able to detect any structure in it. When the stain is over-extracted it appears to come first out of the periphery of the nucleus and last of all out of the central part, but this appears to me to be merely the expression of the dense texture of the kinetonucleus, and not to indicate any structural difference between central and peripheral regions. After the Romanowsky stain the kinetonucleus appears very much larger than it does after iron-hæmatoxylin; it may be four or five times as large (compare figs. 33–36 and 57–64; 40–43 and 66–72; 1–7 and 78–85, &c.). As I have stated above, I believe this result to be due to the fact that the stain forms a deposit round it. With iron-hæmatoxylin the size of the kinetonucleus is quite uniform if the stain be not over-extracted, in which case also the true size is indicated by a clear space surrounding the central portion stained black.

The blepharoplasts appear usually as very minute dots, scarcely thicker than the flagellum, after iron-hæmatoxylin. There appears to be a band of fibril connecting the kinetonucleus and the blepharoplasts, which is shown by iron-hæmatoxylin, at a certain degree of extraction (fig. 98); it stains much less deeply than the kinetonucleus. After the Romanowsky stain the blepharoplasts often appear much larger and may be conspicuous; hence this stain is useful for demonstrating their existence, although it

* I adhere, in my descriptions of the structure of these parasites, to the terminology of the organs suggested by me in the 'Quarterly Journal of Microscopical Science,' lii. pp. 171–174, text-fig. A; with the difference that I employ the term "karyosome" for the intranuclear body there termed "centrosome." To judge by its behaviour during nuclear division, the karyosome of a trypanosome probably contains a true centrosome or "centriole."

does not exhibit them in their true proportions. The flagella also share in the general enlargement after the Romanowsky stain, and appear very much thicker than they do after iron-hæmatoxylin, which shows them as very delicate filaments; they give up the stain more readily than do the true chromatic structures.

The trophonucleus is very different in its structure from the kinetonucleus. After iron-hæmatoxylin (Plates IV. and V.) it appears as a clear space, oval or round, limited by a faint but quite definite membrane, which, when carefully examined, appears granular and uneven in thickness, and is probably composed of granules of chromatin connected together; it is therefore not a true nuclear membrane, in the sense in which the term is used for Metazoan nuclei. The trophonuclear membrane is much less distinct in *Trypanoplasma* than in *Trypanosoma*; a difference perhaps largely due to its being obscured in the former genus by the numerous and deeply staining cytoplasmic granulations. In the space enclosed by the nuclear membrane lie one or more karyosomes, rounded bodies often very large, and staining very deeply with iron-hæmatoxylin. The karyosomes appear to me to be simply masses of chromatin; they retain the stain very tenaciously. Typically there is a single karyosome placed more or less centrally, but their size, number, and arrangement vary greatly, as will be seen by reference to the figures and special descriptions of the species. In the space between the karyosome or karyosomes and the nuclear membrane there are found minute chromatin granules, often disposed so as to leave a clear space round the karyosome; the granules themselves are often so minute as to be scarcely visible.

Where, it may be asked, are the definite chromosomes, eight in number, surrounding the karyosome, so often described by Schaudinn, Léger, Keysselitz and others? Where indeed! I can but describe what I have seen, and I have never seen, after iron-hæmatoxylin, more than I have described above. Possibly the Schaudinnian chromosomes make their appearance at certain stages of the development or phases of the life-history. I can only say that they have never revealed themselves to me in any of my preparations. After the Romanowsky stain, it is true, a great variety of appearances can be seen, impossible to interpret in a uniform manner: sometimes the whole trophonucleus appears an even red mass, sometimes it shows coarse granulations disposed in various ways; in all cases its great difference in size, structure, and appearance from what is seen in the iron-hæmatoxylin preparations is quite bewildering. I have put forward above, in the section dealing with technique, what I believe to be the explanation of this. I hope to discuss the whole question much more fully in dealing with *Trypanosoma lewisi* elsewhere.

Lühe has given * two figures of the trypanoplasm of the carp stained with iron-hæmatoxylin. He figures the trophonucleus as

* In Mense's 'Handbuch der Tropenkrankheiten,' iii. p. 83, fig. 5.

a simple black rounded mass. I am of opinion that what he has figured is simply the karyosome, and that the peripheral portion of the nucleus is not shown. The nuclear space and membrane are often not at all distinct, as I have said above, and I have often seen the trophonucleus appearing just as Lühe has figured it.

In the cytoplasm there are commonly numerous coarse granulations, which stain like chromatin after Romanowsky, but I feel strong doubts as to their being chromidial in nature. After iron-hæmatoxylin I found that the granules gave up the stain much more readily than the true chromatic structures, in the trypanosomes; but in the trypanoplasm of the pike they held the stain very tenaciously. Their true nature could only be determined by the development.

In the trypanosomes of the perch (figs. 96, 97) and eel (fig. 84) I was able to obtain preparations showing the myonemes after the iron-hæmatoxylin stain. I have not been able to see myonemes in any trypanoplasm. I am convinced that to see the myonemes it is necessary to get just the right degree of extraction of the stain; a shade less extraction, and the myonemes are obscured by the darkness of the cytoplasm; a shade more, and the myonemes give up the stain. The darkly staining, opaque cytoplasm of the trypanoplasms probably makes it much more difficult to render visible the myonemes than in the case of the trypanosomes. From my preparations the myonemes appear to be about eight in number in the trypanosome of the eel, but more than that in the trypanosome of the perch. I have never been able to make them out with certainty after the Romanowsky stain.

With regard to the question of the transmission of the trypanosomes and trypanoplasms of fishes, on which much light has been thrown by Brumpt and Keysselitz, I have only a few negative results to record. I found leeches very seldom on the fish caught by me; only once on a rudd, and once on a perch, a *Piscicola* was found attached. On the other hand almost every fish, of whatever species, had *Argulus* attached to it when caught; sometimes there would be five or six *Arguli* on one fish. *Argulus* is stated in all the memoirs and text-books dealing with it to feed on the blood of fishes; it is an active swimmer, and readily leaves one fish and attaches itself to another. It seemed to me therefore that *Argulus* was a creature admirably suited by its habits to disseminate blood-parasites, either by the direct mechanical method, or with a cycle of development. Moreover, *Argulus* is beautifully transparent, and everything in its interior can be seen in the living animal under the microscope; it is easy to focus its blood-corpuscles flowing through the heart and circulating in all parts of its body-cavity, or to see all the contents of its digestive tract, without injuring the beast in any way. It would be quite an ideal form in which to study the development and transmission of hæmoflagellates. I took numerous *Arguli*, kept them hungry for

a day or two, and then put them into bell-jar aquaria containing various fishes. As a rule the *Arguli* attached themselves at once to the fish very firmly. After leaving them for varying periods I removed the *Arguli* and examined them, first living and uninjured under the microscope, and afterwards dissected in salt-solution and examined fresh. Although the fishes on which the *Arguli* fed contained trypanosomes or trypanoplasms in all cases, I never found a flagellate of any sort or description in the *Arguli*, however the experiment was varied; and, moreover, I never in any case found the *Arguli* to contain blood-corpuscles. It might be supposed that an animal so transparent as *Argulus* would show the effects of a meal of blood by a change of colour visible to the naked eye; but in no case could a change of colour be discerned by any optical means whatsoever. I very much doubt, therefore, if *Argulus* feeds on blood, or at least on blood-corpuscles. Very possibly only fluid plasma or lymph can pass the very minute terminal aperture of its proboscis.

My experiments with leeches were greatly hampered by the fact, in the first place, that I can lay claim to no special knowledge of the Hirudinea, and secondly, that no general monograph of this group was accessible to me of later date than that of Moquin-Tandon (1846). It would be a great boon to those studying these parasites if a more recent monograph or handbook were in existence. It is not every student of hæmoflagellates who is at the same time, like M. Brumpt, a first-class expert on leeches. I obtained a few specimens of *Piscicola*, but found nothing in them of flagellate nature. I put a *Piscicola* in an aquarium in company with a perch; the leech took no notice of the fish, nor the fish of the leech. After some days I removed the leech, dissected it, and found nothing in it. I also caught in the Broad some other leeches, which I could not identify accurately, and put them in with perch; in all cases the leeches vanished completely, and were apparently eaten by the fish. *Voilà tout*!

I can therefore make no positive additions to the transmission-question, but I hope to return again to this point when opportunity offers itself.

Description of the Species.

1. Trypanosoma percæ Brumpt. (Plate I. figs. 8–15; Plate V. figs. 94–104.)

Brumpt (CR. Soc. Biol. lx. 1906, p. 161) describes this species as follows:—"Dans le sang de la Perche (*Perca fluviatilis*). Ce parasite mesure 57μ de longueur totale, dont 16μ pour le flagelle, et 3μ de large. Le blépharoplaste est à 1μ·5 de l'extrémité postérieure. Le noyau se trouve à égale distance de la racine du flagelle et de la partie postérieure."

I identify the trypanosomes found by me in the Perch as *T. percæ* Brumpt solely on the ground of occurrence, since Brumpt's description might apply to almost any trypanosome, and fails to

note any of the peculiarities of the trypanosome of the perch, or to take into account its variations of size and structure.

Trypanosomes were found more or less abundantly in almost all the perch examined by me. In a few of these fish none was seen, or they were found only after more prolonged searching; as a rule, however, they were found at once and were present in considerable numbers. If a fish was not well infected, I did not trouble to make smears of it or to examine it further. Hence the few cases in which I did not find trypanosomes were probably cases in which careful search would have revealed the presence of the parasites in scanty numbers. I doubt if any of the perch were really entirely free from them. From the perch I examined, I gained the impression that the trypanosomes were rather more abundant in small or medium-sized fishes than in those of the largest size. I did not find trypanoplasms in any of the perch examined *.

Examination of the blood freshly drawn from the perch showed that the trypanosomes differed considerably in size. They may be divided, speaking generally, into large and small forms. The large forms were much the most abundant; the small forms were very scarce. The large forms when seen living (figs. 94, 95) appeared stout and sluggish, as a rule of considerable size, but showing marked variations in this respect, some being smaller and more active. They wriggle incessantly but do not travel much. The body is spindle-shaped; one end, which bears the flagellum, is greatly attenuated and sharply pointed; the other is also drawn out, but is much less attenuated and appears to end more bluntly. At the blunt end a distinct, very refringent dot could always be seen, doubtless representing the kinetonucleus; it appeared light at a high focus, black at a low focus. Sometimes two small dots could be seen (fig. 94), in which case the second was probably the blepharoplast. The nucleus could be seen distinctly as a rounded clear space in which the karyosome appeared as a darker spot, not very refringent. A short way behind the nucleus there was seen in some specimens a distinct dot or grain, much more refringent than the karyosome, and apparently lodged in a clear space or vacuole (fig. 95); this body was not always seen. The undulating membrane and flagellum could be clearly distinguished, especially when the parasites were moribund and becoming slower in their movements, after being under observation for some hours.

The trypanosomes show a great tendency in the living state to twist and roll themselves up, in a way that calls to mind a snake of stout build, such as a python. Their movements were of two kinds, which have been distinguished above as wriggling and travelling movements respectively. When wriggling they simply twist over and over, throwing themselves into S-like curves, without changing their place. When travelling the body is

* Keysselitz (Arch. Protist. vii. 1906, pp. 2, 3) records the occurrence of both trypanosomes and trypanoplasms in *Perca fluviatilis*.

stretched out, with the undulating membrane rippling actively, the undulations commencing at the flagellar extremity and passing backwards down the body. They travel usually with the flagellum forwards and the body straightened out; the movement is effected apparently, chiefly by the undulating membrane; the blunter posterior prolongation of the body meanwhile performs curling or wagging movements which appear to be of a passive kind, the result of the movements of the undulating membrane. I saw a trypanosome, travelling in this manner on a slide under a coverslip, approach and go straight through a closely packed mass of blood-corpuscles, flagellum foremost and body straight. The same trypanosome was seen also to reverse its movement and move with flagellum directed backwards; the blunter extremity of the body, now foremost, performed movements which appeared to be active in nature, and to assist the body in penetrating forwards. This was observed also in other cases. Trypanosomes which were travelling actively were observed to come to a standstill and perform simply wriggling movements; conversely, trypanosomes which have been wriggling for some time may start off and begin travelling.

The smaller forms were very similar to the large forms in their general appearance and the character of their movements, and only differed in their small size, pronounced slenderness, and much greater activity and rapidity of movement.

The trypanosome of the perch does not seem to possess the extraordinary vitality of *T. granulosum* of the eel. I found that the trypanosomes did not live more than a few hours *in vitro*. I made an observation, however, relating to their vital powers which perhaps is not without interest. From a perch which was very well infected, and from which I made a number of smears (fig. 11 was taken from this fish) I took three drops of blood; one drop was simply placed on a clean slide and covered with a cover-glass; the second drop was placed on a slide and mixed with a drop of Laveran's salt-citrate solution, and then covered; the third drop was put on a slide and mixed with a drop of tap-water, and then covered. The three drops were studied from time to time. In the drop mixed with water the trypanosomes showed greatly increased activity after about an hour, travelling with great rapidity in the hæmolyzed blood; after four hours they were still very active, but not quite so active as they had been earlier. After five hours I slipped off the coverslip, smeared out the blood, dried it, fixed it with absolute alcohol, and stained it with Giemsa's stain. The trypanosomes stained well and appear perfectly normal. In the drop of blood mixed with salt-citrate solution the trypanosomes also remained active but some of them were changed in form after four hours, the body becoming pear-shaped, with most of its substance aggregated at the hinder end. In some cases the trypanosomes appeared as if anchored by the hinder end; the anterior end of the body lashed round in all directions, but seemed unable to move the inert posterior

mass. After about five hours I slipped off this coverslip also and made a preparation of the blood, but it only shows trypanosomes very badly preserved and stained, and apparently quite degenerate in structure. In the control drop of pure blood the trypanosomes were all dead or moribund after four hours, and I did not proceed further with it. If any conclusion can be drawn from these experiments, it is that the trypanosomes live, and remain normal, longer when the blood *in vitro* is mixed with water than when it is mixed with salt-citrate solution or left pure; a result which I certainly did not expect.

In preparations the trypanosomes of the perch show a continuous gradation of sizes from the smallest to the largest (figs. 8–14); the larger forms being, however, by far the commonest. The best means of classifying them is by the free flagellum, which in the large stout forms is very short (figs. 13, 14), but in the medium-sized (fig. 12) and small forms (fig. 8) is much longer. The cytoplasm stains a very deep blue with the Giemsa stain, so deeply in fact that it is very difficult to obtain satisfactory preparations of the stout forms; they appear often as bluish opaque masses in which the intensity of the stain obscures all details of structure. In the same preparations, on the other hand, more slender forms may be found perfectly stained. The cytoplasm in the largest forms usually appears blotchy, with lighter and darker parts, often with tiny vacuole-like spaces, not very sharply limited. Any of the forms may contain red-staining granules to a greater or less extent, sometimes very numerous, sometimes absent altogether. The granules in question are of fair size and more or less irregular in form. In one specimen I saw them frequently in pairs, and sometimes rod-shaped, suggesting division (fig. 104); and the idea occurred to me that they might perhaps be intrusive organisms of the nature of Bacteria. In never-dried preparations stained with iron-hæmatoxylin the cytoplasm appears more or less evenly and coarsely granular, according to the degree of extraction of the stain (figs. 96–99). The above-mentioned granules are not brought out by this stain. I see, therefore, no reason for regarding them as chromidia.

The nucleus appears, in the smears stained by Giemsa's method (figs. 8–14), as a red patch, often obscured and difficult to make out clearly in the large stout forms. It varies in size with the dimensions of the trypanosomes, and is much larger in the large forms. With the Romanowsky stain the details of nuclear structure appear to vary greatly; sometimes a distinct sharply limited karyosome, stained a deeper red than the rest of the nucleus, can be made out (fig. 13), sometimes not. It would appear as if the method of drying had the frequent result of distorting or breaking up the fluid or plastic karyosome, thus producing different appearances in different cases. On the other hand, in never-dried smears stained with Heidenhain's iron-hæmatoxylin, the structure of the nucleus appears quite uniform in all its principal features (figs. 97–102), in all cases, and can be

described in quite general terms. It is seen that the nucleus is an ovoid or nearly spherical space limited by a delicate membrane. In the interior is a deeply staining karyosome, which may be spherical, ovoid, pear-shaped, or even dumbbell-shaped. The karyosome is always large, and sometimes so large as to nearly fill up the entire nuclear cavity and appears to be immediately surrounded by a clear space. The remainder of the nucleus is occupied by a faintly granular material, which, owing to the excentric position of the karyosome, forms usually a crescentic area on one side of the nucleus. In this area coarser dots of chromatin can be made out, especially in the neighbourhood of the nuclear membrane, which is probably composed also of chromatin. No details of structure could be seen in the karyosome itself.

The foregoing statements apply to the nuclear structure of the large or medium-sized forms, since I was not able, unfortunately, to find any of the small forms, always rare, in my preparations stained with iron-hæmatoxylin.

The kinetonucleus appears as a rounded or ovoid mass in preparations stained by the Romanowsky method. It is larger, both absolutely and relatively, in the very small trypanosomes than in the large. In preparations stained with iron-hæmatoxylin it appears either rod-shaped or rounded in form, but in either case very much smaller in size than it appears when stained by the Romanowsky method. If the hæmatoxylin be not much extracted, the kinetonucleus is often difficult to distinguish from the blepharoplast, the two together appearing to form a single mass of triangular or irregular outline. If, on the other hand, the extraction of the hæmatoxylin be carried too far, the blepharoplast and flagellum become completely decolorized and the kinetonucleus appears as a very sharply defined and deeply stained body, in which no details of structure could be distinguished.

The flagellum arises from a distinct blepharoplast, which can almost always be seen clearly, and usually lies close beside the kinetonucleus. In some cases, however, the blepharoplast and kinetonucleus are separated by a considerable interval, and then appear to be connected by an ill-defined band of material staining more lightly (fig. 98). In the living condition also, as stated above, I observed in some cases two separate grains, which apparently represented the kinetonucleus and blepharoplast.

The flagellum arises direct from the blepharoplast and runs along the edge of the undulating membrane in the usual way. As already stated, the free flagellum is short in the large stout forms, long in those of medium or small size. It is worthy of note that in the smaller forms the free flagellum ends distally in a distinct grain or dot. The undulating membrane is shallow, with many small pleats as a rule, in the stout forms with short free flagellum (fig. 13); but in all forms with a long free flagellum,

and especially in the smallest forms, the undulating membrane is very deep, and stands out far from the body, with fewer and larger pleats (figs. 8–12). It generally appears clear, but in some cases the granules of the cytoplasm can be seen extending up into it, forming a distinct contour-line close under, but quite separate from, the flagellum (fig. 12).

In one of my preparations, which had been fixed first with osmic vapour and then with Schaudinn's fluid, without drying, and stained with iron-hæmatoxylin, I found the trypanosomes showing distinctly striations which are doubtless to be explained as myoneme filaments (figs. 96, 97). They are to be made out on both surfaces of the body running spirally, and hence appear to cross in the drawing, but in the object they are seen at different foci on the two surfaces. In one trypanosome they appear to run in couples (fig. 96). In another, which was much bent up and probably contracted, the myonemes can be seen on one surface running to a very convex edge, where they appear in optical section as distinct grains, and from this point they can be traced again on the other surface (fig. 97). The exact number was difficult to make out; fig. 96 indicates that there are in all 8, or 4 couples, but in fig. 97 there appear to be more than this. To these myonemes may be referred the active wriggling movements of the trypanosomes.

In some of my preparations stained with Giemsa's stain I found very broad forms of the trypanosome. I am convinced that these forms are simply stout forms of the trypanosome deformed and flattened out by drying. In slides fixed with osmic vapour before drying I find them only at the edge, or in very thin parts of the film, that is to say in places where it is difficult to avoid a slight amount of drying taking place. Two other points are to be noted in favour of this conclusion; one is that the very broad forms are not so opaque as the stout forms which do not show any flattening; the other is that the nucleus is more or less considerably elongated in the transverse direction, having evidently shared in the increase of breadth produced by the flattening.

In one preparation, fixed wet with osmic vapour and stained by Giemsa's method, I found a trypanosome apparently encysted (fig. 15). No trace of a flagellum was to be seen, but the body is rounded off and surrounded by an envelope staining a faint bluish tinge. The shape of the nucleus indicates perhaps that a slight amount of flattening has taken place. No other similar stage was found.

In preparations from a perch which showed abundant trypanosomes in its blood, I found, on two separate slides, two bodies resembling hæmogregarines (fig. 16); they were free in the blood-plasma and strongly resembled the free vermicules of these parasites. It is well known that hæmogregarines occur commonly in marine fishes, but in freshwater fishes they have only been found in the eel. I searched long, but in vain, for intracorpuscular

stages similar to the vermicules; I found, however, in the large uninucleate* leucocytes, bodies which I took at first to be parasites: rounded bodies staining a faint pink (after Giemsa's stain), with a central darker grain or two such grains (figs. 17–19). I could not find, however, anything in the least transitional to the vermicule-like bodies, and I do not think now that the bodies in the leucocytes are of parasitic nature. In the corresponding leucocytes of the tench (figs. 45–48) there are to be found commonly, but not invariably, round pink-staining bodies, sometimes a single one of variable size, sometimes two, three, or four such; they are clearly vacuoles containing some substance which has stained, probably, with the eosin of the Giemsa stain, and I think it very probable that the bodies in the leucocytes of the perch are of a similar nature.

If the vermicules, however, are not stages of a hæmogregarine, in what light are we to regard them? Since they were found in the same blood as the encysted trypanosome already described (fig. 15), the idea occurred to me that perhaps the stout forms of *Trypanosoma percæ* might encyst in the internal organs and undergo multiplication to form the vermicule-like bodies; these in their turn might acquire flagella and so give rise to the smallest forms of the trypanosome, which by growth into the large forms would complete a cycle of multiplication in the fish. The rarity of fission-stages of the trypanosomes of fish is remarkable; I have never seen a fish-trypanosome in division, but Laveran and Mesnil have described fission of *T. remaki* in two pike infected artificially, and Lebailly and França have, as stated elsewhere, described fission in *T. granulosum* from cultures *in vitro*. It is therefore quite possible that fission may be a process restricted to certain parts of the life-cycle, and that the usual mode of multiplication in fish-trypanosomes may be such as I have indicated above. In *Trypanosoma lewisi*, for example, fission is only found during the first week or so after inoculation; there is then no further multiplication, the trypanosomes being all of one size and type. On the other hand, fish-trypanosomes usually exhibit marked variations in size which are very suggestive of growth from the smallest to the largest forms. I desire to make this suggestion cautiously, as the data on which it is founded are obviously quite inadequate to establish it. I may point out, however, that the blood in which the encysted trypanosome and the vermicule-like bodies were found, was taken from the heart of the fish with a capillary glass tube, and it is quite possible that the tube in passing through the walls of the heart may have taken up bodies which were not free in the general circulation, but contained in the wall of the heart itself.

* Commonly termed "mononuclear"; a barbarous etymological compound. The adjective "nuclear" means "of or relating to the nucleus"; not "possessing a nucleus."

2. Trypanosoma granulosum Lav. & Mesn. (Plate I. figs. 1–7; Plate V. figs. 78–93.)

The trypanosome of the Eel has been seen by many observers, and there is no other fish-trypanosome which has been the object of so many memoirs. According to Laveran and Mesnil, the first description of this parasite was by Sabrazès and Muratet, but the earlier work of these authors is not accessible to me. The earliest memoir on the subject with which I am acquainted is that of Laveran and Mesnil themselves (1902), in which they figure and describe the parasite and name it *T. granulosum*. Lebailly (1906) gave a detailed description, with two figures, of this trypanosome and distinguished two varieties, *magna* and *parva*. In the same year Brumpt described the transmission of the trypanosome of the eel by the leech *Hemiclepsis*, and the development that the parasite goes through in this leech. Finally, França (1907) has devoted a memoir to this trypanosome.

Since so much work has been done on the trypanosome of the eel, I did not pay much attention to it, not expecting to be able to add much to our knowledge on this subject. I obtained four eels caught in the Broad and examined one of them, which I found to contain trypanosomes most abundantly, more so than any fish I have yet examined. I made a number of smears and preparations from this eel and then sent all four eels to the kitchen, to be prepared for my next meal; it was not found that infection with trypanosomes impaired appreciably their gastronomic properties. I much regretted afterwards that I did not examine the other three eels to see if they were equally well infected.

Sabrazès & Muratet (1904), Lebailly (1906), and França (1907), have all drawn attention to the extraordinary vitality of this trypanosome, and the fact that if a drop of blood containing them be sealed up on a slide under a coverslip, the trypanosomes will live for several days and multiply. I have not imitated these experiments, but I had a drop of blood under a coverslip, not sealed up in any way, in which the trypanosomes were moving actively after 24 hours, less actively after 48 hours, and feebly after 72 hours.

I spent some time watching the movements of this trypanosome, which are extraordinarily snake-like. As in the case of *T. percæ*, the movements are of two kinds, conveniently distinguished as wriggling and travelling. They may wriggle for a long time without changing place to any considerable extent, and then they may suddenly start travelling. When they become weaker, after 24 hours under a coverslip, they only wriggle and do not travel, and as they become moribund the wriggling movements become weaker and weaker, till on the third day they are very feeble. When wriggling they twist over and over in S-like curves, appearing at the first glance like a writhing knot. When travelling, I observed them always progressing actively in a definite direction, flagellum forwards, the body twisting from

side to side in even curves like a snake. They frequently stop suddenly, and wriggle actively in one place like an excited earthworm, and then start off again in a new direction. I never saw them travel with the flagellum directed backwards. The undulating membrane is very distinctly seen, and shows beautiful rippling movements. Progression appears to me to be effected chiefly by twists and turns of the body, aided doubtless by the undulating membrane; the flagellum has perhaps chiefly a tactile or guiding function. The kinetonucleus is very distinct in the living condition as a refringent granule a short way from the hinder end. The trophonucleus is more difficult to see during life.

Although my preparations were made only with the object of adding to my collection, they show some points of interest, which I will state briefly.

In the first place my preparations show trypanosomes of all sizes from very small to very large (figs. 1–7). The contrast between the two extremes of the series is surprisingly great, but all possible transitions from one extreme to the other are to be met with. Nor can I find any definite structural features to separate sharply the large and small forms, as can often be done in trypanosomes of other species. The large forms are very granular, while in the smallest granules are absent or scarce, but this feature shows both variations and transitions; the large forms have more pleats in the undulating membrane than the smaller, but the pleats are about the same depth and simply increase in number with the length of the trypanosome. The free flagellum bears about the same proportion, as regards length, to the rest of the body in both large and small forms, though in some large forms it is perhaps relatively shorter. The differences in the nucleus, to which I shall refer presently, are also differences due to a gradually increasing complexity of structure. I cannot therefore find characters to separate sharply the varieties *magna* and *parva* of Lebailly.

A very marked feature of this species, when stained by the Romanowsky method, is the occurrence of numerous granules in the cytoplasm, a peculiarity to which it owes its name. The granules stain purple or blue with Giemsa's stain. In preparations stained with iron-hæmatoxylin, the granules are only to be seen when the stain is not sufficiently extracted (fig. 83); when the stain is most satisfactory and the flagellum and nuclei are sharp and distinct, the characteristic granulations are not to be seen. One of my coverslip-smears is rather uneven and thicker in some parts than in others. In the thicker portions the stain is less extracted than in the thinner parts of the smear, as commonly happens, and hence different degrees of extraction can be found in the preparation. Only a very few trypanosomes show the granules stained and standing out sharply; with too slight extraction of the stain the granulations are obscured by the darkness of the cytoplasm. If the stain is very much over-

extracted, as in one of my preparations, the colour comes out of everything except the kinetonucleus; when the extraction has not gone quite so far, the karyosome of the trophonucleus also retains the black colour. The granulations of the cytoplasm are not, in my opinion, to be regarded as chromidia, since they give up the stain very readily, while the true chromatic structures retain it very tenaciously.

França has drawn attention to the peculiar structure of the nucleus in the variety *magna* of this trypanosome. He writes:— "Le noyau ..... s'éloigne par sa structure de celui de presque tous les Trypanosomes. Au sein d'une substance incolore on voit la chromatine formant d'ordinaire deux parties bien distinctes; un grand bloc ..... d'un rouge vif, situé dans la partie du noyau la plus rapprochée du blepharoplaste; et un grand nombre de granulations petites et régulières, ou un reticulum chromatique, vers le coté du flagelle. Dans quelques parasites, plus rares, on ne voit pas le grand bloc de chromatine et alors il existe dans la substance nucléaire incolore une série de granulations disposées de façon à former un cordon plus au moins sinueux." "Au contraire de ce qui existe dans le noyau des Trypanosomes de la var. *magna*, dans ceux de la var. *parva* il n'y a pas une distinction nette entre les portions chromatique et achromatique."

França's observations appear to have been made on trypanosomes stained by the Romanowsky method, and my preparations coloured with Giemsa's stain confirm his statements. But I do not believe it is possible to get a coherent or intelligible idea of the structure of the nucleus with this method of staining. My preparations stained with iron-hæmatoxylin show appearances quite different from those seen after use of the Romanowsky stain, and at the same time permit of a uniform interpretation of the structural details. The nucleus appears very much smaller and more compact after iron-hæmatoxylin than after the Romanowsky stain; and while it is possible that there is a certain amount of shrinkage by the former method, I think there is certainly a considerable amount of artificial expansion and deformation consequent on the process of drying by the ordinary method of applying the Romanowsky stain.

In never-dried preparations stained with iron-hæmatoxylin, the smallest trypanosomes (figs. 78, 79) show the nucleus as a clear oval space with a distinct limiting membrane, containing a sharply defined karyosome of elongate-oval form. The karyosome is far from filling up the entire nuclear space, which appears clear or shows very minute granulations. A nucleus of this type of structure may be found even in trypanosomes of large size (fig. 87), but the karyosome in such specimens is considerably larger and fills up nearly the whole nuclear cavity. As a rule, however, in trypanosomes only slightly larger than the smallest that can be found (figs. 80, 81), the karyosome is seen to have budded off from one extremity, usually from that furthest from the kinetonucleus, a smaller part. This is the type of nucleus